Temperature, irradiation and delivery as factors affecting spring-time flight activity and recapture of mass-reared male codling moths released by the Okanagan-Kootenay sterile insect programme

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ABSTRACT

Laboratory flight-tunnel and field mark-release-recapture experiments were conducted to compare pheromone response, flight activity and recapture of wild codling moths, *Cydia pomonella* (L.), with codling moths mass-reared by the Okanagan-Kootenay Sterile Insect Release Programme. These experiments were designed to identify factors that may contribute to poor pheromone trap catches of sterile moths in the spring. Irradiation (250 Gy) had no influence on catches of mass-reared moths in pheromone traps at spring (16 °C) or summer temperatures (25 °C) in flight-tunnel assays. In field experiments however, recapture of mass-reared and wild moths in pheromone traps was significantly reduced after irradiation, suggesting effects of irradiation were modified by additional factors acting in the field. Catches of mass-reared moths in flight-tunnel assays showed a nonlinear increase with increasing temperature. There was no evidence that mass-reared moths were less responsive to pheromone at low temperatures than wild moths. Based on *x*-intercepts of linear regressions of percent catch *vs.* temperature (15 – 25 °C), flight-temperature thresholds for mass-reared (14.7 °C) and wild moths (15.4 °C) were similar in flight-tunnel assays. Irradiated moths carried for 4 h on all-terrain vehicles used for delivering sterile moths were less responsive to pheromone lures in subsequent flight-tunnel assays than moths that spent no time on these vehicles, but only when flown at spring-like temperatures (16 °C). In field tests, moths released on the ground were caught significantly less often than moths released within the tree canopy and negative effects of ground release appeared greater when made in spring compared with autumn.

Key Words: Codling moth, sterile insect technique, sterile:wild ratios, flight-temperature thresholds, flight tunnel tests, mark-recapture tests

INTRODUCTION

The Okanagan-Kootenay Sterile Insect Release (SIR) Programme was initiated in 1992 to eradicate codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), from montane fruit-growing regions in British Columbia (BC). Dyck *et al.* (1993) designed this SIR Programme with three phases: (1) pre-release sanitation (two years), (2) sterile moth release (three years), and (3) surveillance monitoring and protection (open-ended). The objective of phase 2 was to deliver sufficient sterile moths each week to maintain ratios of ca. 40 sterile (S) to 1 wild (W) male moth in pheromone trap catches for the entire season. This 40:1 ratio was deemed necessary if sterile moths were going to reduce wild populations to near extinction in three years (Proverbs *et al.* 1982, Dyck *et al.* 1993).

Following a pre-release sanitation pro-

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gramme that extended from Osoyoos to Summerland and included the Creston and Similkameen Valleys (49° 34′ N Latitude - 119° 39′ W Longitude), sterile moths were released area-wide in May 1994. SIR Programme trapping data (1994 - 2004) indicates that since 1994, S:W ratios have rarely reached 40:1 in the spring, often failing to reach 10:1, whereas target ratios were usually achieved in the summer (Thistlewood *et al.* 2004). Consistently low S:W ratios in the spring have delayed population suppression, made supplementary controls necessary and increased programme costs (Thistlewood and Judd 2003, Judd *et al.* 2004, Judd and Gardiner 2005). In recent years the focus of the programme has changed from eradication to management, but because sterile moths continue to be the primary control tactic in spring, improvements in programme delivery are needed to make it economically sustainable (Dendy *et al.* 2001). Understanding the factors that contribute to inactivity of sterile moths in the spring may lead to corrective action and improve the economics of the programme.

Bloem and Bloem (1996) hypothesized that cool weather was largely responsible for suboptimal S:W ratios in the spring, implying mass-reared moths fly poorly at low temperatures. Although normal seasonal increases in temperature and recapture rates of sterile males are correlated, a clear cause and effect relationship between temperature and flight activity of sterile moths has never been demonstrated (Judd *et al.* 2004). In nearly all studies where the activity of sterile moths in relation to temperature has been discussed, catches in pheromone traps have been used to measure this activity (Hutt 1979, Rogers and Winks 1993, Bloem *et al.* 1998, 1999, 2004, Judd *et al.* 2004). Interpreting these data is difficult because several factors are confounded. For example, mass-reared codling moths may fly poorly at cool temperatures, but it is equally plausible that mass-reared moths have undergone behavioural changes related to pheromone communication that are only expressed under cool spring temperatures. Also, as none of the above studies measured the relative effects of irradiation or ground release on trap catches, or included similarly-treated wild moths, any adverse effects of mass-rearing can not be separated from interacting effects of irradiation, handling and release techniques.

Our objective was to identify factors that might contribute to poor activity of sterile moths in the spring in an effort to take corrective action to improve S:W ratios in the operational SIR Programme. We undertook studies to specifically examine the effect of air temperature on pheromone response of mass-reared codling moths relative to wild moths and to determine if temperature modified any effects of irradiation and handling. In this study we use field mark-release-recapture tests to assess activity of codling moths (Bloem *et al.* 1998), but also use a laboratory flight tunnel because we wanted to isolate effects of temperature on activity and pheromone response without the confounding effects that field experiments impose.

MATERIALS AND METHODS

Test insects. Wild codling moths used in these experiments were collected as diapausing larvae from several organic apple orchards in the Similkameen Valley. Corrugated cardboard bands were wrapped and stapled to trunks of apple trees in July to capture overwintering, diapausing fifth instar larvae (Judd *et al.* 1997). Bands were removed from orchards in early October and transferred to an outdoor screen house at the Pacific Agri-Food Research Centre (PARC) in Summerland. They were held there in plastic garbage bags until March of the following year, when they were placed in a 0.5 °C growth chamber in total darkness. Wild larvae were brought out of cold storage as needed for experiments and set up in emergence cages held in environmental chambers at 27 °C under a 16:8 h Light:Dark (L:D) photoregime.

All mass-reared codling moths used in these experiments were produced by the Okanagan-Kootenay rearing facility in Osoyoos, BC as described by Bloem and Bloem (2000). For experiments requiring non-irradiated moths, trays of artificial diet (Brinton *et al.* 1969) containing mature larvae were provided by the Osoyoos rearing facility as needed and transferred to an environmental chamber at PARC where they were held at 27 °C under a 16:8 h L:D photoregime. Mature pupae were removed from the diet, sexed and placed individually in 30 ml plastic cups provided with wet cotton wicks until moths eclosed. Male and female moths from all sources were isolated in separate environmental chambers maintained at 27 °C and 65% relative humidity with 16:8 h L:D photoregime before testing.

Irradiated, mass-reared moths were obtained from the SIR Programme's Osoyoos rearing facility. Moths were collected in adult emergence rooms (27 °C) after flying out of diet trays towards UV lights located on the ceiling. Vacuum hoods adjacent to UV lights drew moths through pipes into a collection room maintained at 2 °C. Chilled moths were then packaged by weight into plastic petri dishes in which they were irradiated. Moths were sterilized by exposure to 250 Gy (11.5 - 13.2 Gy min^{-1}) of gamma radiation from a $Cobalt^{60}$ source (Gammacell 220, Nordion, Canada). After irradiation petri dishes were loaded into a refrigerated trailer (4 °C) and trucked to area drop-off points. There they were placed either in temporary storage facilities (4 °C) awaiting pickup by delivery drivers, or directly into coolers (6 - 8 °C) on the back of all-terrain vehicles (ATVs) outfitted with moth-dispensing units (McMechan and Proverbs 1972). Irradiated moths destined for release were moved from ATV coolers and placed in a small hopper on the front of the ATV, where a small fan unit dispensed moths by gently blowing them onto the ground beneath trees. In some cases sterile moths spent up to 4 h in the release-vehicle cooler before being dispensed at the end of a delivery route. Moths used in this study were collected after delivery to field cold-storage units, or after being carried by drivers on moth-release vehicles for 4 h.

Flight-tunnel procedures. A pushing-type flight tunnel described in detail by Judd *et al.* (2005) was used to assess behavioural responsiveness of male codling moths to sex pheromone sources in clean air. An air conditioning unit attached to the air intake vent at the upwind end of the tunnel allowed us to achieve flight temperatures of ca. 10 - 25 °C in the tunnel. Detailed description of moth handling procedures and experimental protocols for flight-tunnel assays are described by Judd *et al.* (2005). Pheromone lures used in flight-tunnel experiments were made from red rubber septa (Aldrich Chemical Company Inc., Milwaukee, Wisconsin) loaded with 200 µl of dichloromethane containing 10 µg of the codling moth sex pheromone (*E*,*E*)-8,10-dodecadien-1-ol, known as codlemone (99% isomeric and chemical purity, Shin-etsu, Fine Chemicals Division, Tokyo). Septa were air dried for ca. 18 h at 23 °C in a fume hood and stored in sealed jars at 0 °C until used.

Mark-release-recapture techniques. Before each field release and some laboratory assays, moths were chilled for 10 min in a cold room (0.5 °C) and dusted lightly with Day-Glo® Daylight Fluorescent Powders (Switzer Brothers Inc., Cleveland, Ohio, USA). Different coloured powders were used to distinguish groups of moths treated, handled or released differently. Marked moths were placed in plastic petri dishes or 60 ml plastic cups and transported to field sites in ice chests. Dishes or cups were opened in the field and moths took flight under their own capacity. Pherocon 1-CP style, sticky, wing traps (Phero Tech Inc., Delta, BC), baited with similar 10 µg lures as used in flight-tunnel experiments, were used to recapture moths. The 10 µg lure load was chosen because it releases codlemone at a rate similar to an individual female codling moth (Bäckman 1997) and has the advantage of being both attractive in the field, at least for short periods of time, and the flight tunnel, the latter of which is

not true of standard 1 mg field monitoring lures. When experiments were completed traps were returned to PARC where exposure to UV light revealed the fluorescent dusts and moths were counted.

Flight Tunnel Tests

Experiment 1: effects of irradiation on pheromone response. Responses of irradiated (250 Gy) and non-irradiated, mass-reared codling moths to pheromone lures in flight-tunnel experiments were assessed at 16 and 25 °C, temperatures typical of dusk in the spring and summer respectively. On each of seven flight days, uniquely-marked (as above) groups of 9 - 10 irradiated or non-irradiated moths were flown in random order at one of the two randomly assigned temperatures. The percentage of moths caught in a pheromone-baited trap within a 30 min period was recorded, then the other group was flown, after which the temperature was changed and the process repeated. Moth catches were expressed as proportions (p) and transformed using arcsine $\sqrt{p}$. Mean recapture rates for each treatment combination were calculated and compared using a two-way (flight temperature, radiation treatment) analysis of variance (ANOVA) with a temperature × irradiation interaction term in the model. Significance of each factor in the model was tested using an F-test. All statistical analyses were performed with an α value of 0.05 using SigmaStat® (Version 3.0, SYSTAT Software Inc., Richmond, CA).

Experiment 2: effects of air temperature on pheromone response. Pheromone responses of non-irradiated, mass-reared moths and apple-reared wild moths emerging from diapause, were compared in the flight tunnel at 15.5, 17.5, 19 and 25 °C following a randomized block design. On each of 5 flight days (blocks), uniquely-marked groups of 11 - 15 mass-reared and 11 - 15 wild males were flown simultaneously from the same release cage described by Judd *et al.* (2005) at one randomly assigned temperature. The percentages of each moth type caught in a pheromone-baited trap within a 30 min test period were recorded. Temperature was adjusted and the procedure repeated until catch at all four temperatures was evaluated on a given day. Percentage catch for each moth type was plotted against temperature. Linear regression (SigmaStat®) was used to estimate the lower threshold temperatures for pheromone-mediated flight based on the x-intercepts of these lines. A t-test was used to compare slopes of regression lines (Zar 1984).

To verify the lower-temperature flight threshold for mass-reared moths we conducted a set of six additional flights with groups of 17 - 45 mass-reared moths at 13, 14, 14.5, and 15 °C. A one-way ANOVA and Student-Newman-Keuls' multiple comparisons test were used to compare mean (n = 6) percentage capture at each of these temperatures (SigmaStat®).

Experiment 3: effects of handling time in moth release vehicles. Irradiated codling moths were collected at two different points in the moth distribution process used by the SIR Programme. Moths were obtained at noon on each of 10 days after they were delivered by a refrigerated truck and placed in a storage refrigerator at a drop-off depot in Summerland. One petri dish of irradiated moths was removed from the storage fridge and labelled ATV - 0 - h. A second petri dish labelled ATV - 4 - h was removed from a cooler on the back of an ATV which had just returned after four hours of field deliveries. Labelled petri dishes were returned to PARC where moths were sexed and counted in a 0.5 °C cold room. Fifty males were placed in each of several 11.4 L plastic buckets and held overnight at 27 °C in an environmental chamber under a 16:8 L:D photoregime. The following day moths were chilled for 10 min at 0.5 °C, placed individually into release cages described by Judd *et al.* (2005) and transferred to the flight-tunnel room 15 min before scotophase. Following a randomized block design, on each of 10 flight days (blocks), moths from each treatment group (ATV- 0 h and ATV- 4 h) were flown individually in random order at one of two randomly assigned temperatures

typical of spring (16 °C) and summer (25 °C); one moth from each of the two handling times was flown in every two flights. Each moth was placed downwind from a 10 µg pheromone lure and given 2 min to fly upwind and make contact with the lure. After flying 7 - 18 moths from each treatment group the temperature was reset and the process repeated.

On a given test day the percentages of each moth type making contact with the pheromone lure at each temperature were calculated. Percentage data were transformed by arcsine $\sqrt{p}$ and mean rates of source contact at each temperature, for each handling treatment, were compared using a two-way (flight temperature, moth handling treatment) ANOVA with a temperature × handling interaction term in the model. Significance of each factor in the model was tested using an F-test.

Field Tests

Experiment 4: effects of irradiation on pheromone trap catches of mass-reared and wild moths in the field. A mark-release-recapture field experiment was conducted in September to assess the effects of irradiation on rates of moth recapture in pheromone traps. Mass-reared codling moths (from trays of diet provided by the Osoyoos facility) and diapausing wild moths emerged in our laboratory as described in the test insect section above. Two- to three-day-old moths were transported to the Osoyoos rearing facility, where one half of each moth type was irradiated with 250 Gy and the other half remained non-irradiated to serve as a control group. This procedure provided four moth treatment groups: irradiated and non-irradiated, mass-reared and wild moths, respectively. After irradiation, moths were returned to PARC, chilled (0.5 °C) and each of the four moth treatment groups was uniquely marked as before. One moth release device, as described by Judd et al. (2006a), and containing 24 moths of one treatment was hung within the canopy of each of the four corner trees in a 32 × 32 m square release area located near the centre of a mixed-variety apple orchard having a 3 m tree × 4.6 m row spacing and an average tree height of 3 m. Four wing traps, each loaded with 10 µg of codlemone, were hung ca. 1.5 - 2 m above ground in the central tree of this release area. One trap was placed in each cardinal sector of the central tree. After one week, traps were returned to PARC and marked moths caught were identified under UV light. Catches of each moth treatment group in all four traps within a given orchard (replicate) were summed and used to calculate the percentage recapture. This entire procedure was repeated in four independently replicated releases in different orchards. Percentage recapture data were transformed by arcsine $\sqrt{p}$ and analyzed using a two-way (moth type, irradiation treatment) ANOVA model containing a moth type × irradiation treatment interaction term. Significance of each factor in the model was tested using an F-test.

Experiments 5 - 7: effects of ground release on moth recapture. Experiments 5 and 6 were preliminary tests designed to compare the rates of recapture of moths released within the tree canopy with those released on the ground beneath the same trees. Within-canopy release was used to simulate the location that recently-emerged wild moths might likely be found, and ground release was used to simulate the location that sterile moths were delivered by the SIR Programme. Uniquely-marked, irradiated, mass-reared moths were used for these experiments. Paired independent releases were conducted during September in two separate orchards and each orchard release was analyzed as a separate non-replicated experiment (5 and 6). Moths were released and recaptured in 32 × 32 m square release areas exactly as described in experiment 4, with the exception that an additional set of adult moth release devices was placed on bare soil at the base of the four corner trees in each release area. Any effects of moisture in these experiments were minimal because both releases were made during September in an absence of any irrigation and moths were released in early afternoon when dew had evaporated. Direct moth contact with the ground was

minimized by moths flying from release devices under their own power. Within each orchard (experiment), the paired proportions of ground- (p_{GROUND}) or canopy-released moths (p_{CANOPY}) recaptured out of the 128 moths released at each of these locations within each orchard, were compared using *z*-tests on two binomial proportions (Zar 1984).

In experiment 7 the effect of release location on recapture of moths was examined again but under spring temperature conditions in a series of replicated tests. Four independent but simultaneous moth releases (replicates) were made in four similar release areas and moths were recaptured in each area as described in experiments 5 and 6. The experimental sequence was to release moths during afternoons of days 1 - 3 and trap during nights 3 and 4. Traps were removed the morning of day 5 and returned to the laboratory to identify and count moths. The above mark-release-recapture procedure was repeated on four different occasions: (I) 12 - 16 May, (II) 19 - 23 May, (III) 26 - 30 May, and (IV) 2 - 6 June. In total 16 independent releases and recaptures were made. Within each five-day release-recapture test period (I - IV), 128 - 200 uniquely-marked moths were released from within the canopy of four trees and on bare dry soil beneath each of these trees in each orchard. Percentage recapture of moths from each release location (canopy *vs.* ground), within each orchard and time interval was transformed by arcsine $\sqrt{p}$. Recapture data for each time period (I - IV) were analysed separately because each time period represented an independent set of releases rather than a repeated measure on one set of releases. Within each time period mean recaptures from each release location (canopy *vs.* ground) across the four release orchards (replicates) were calculated and compared using a paired *t*-test.

RESULTS

Flight-Tunnel Tests

Experiment 1: effects of irradiation on pheromone response. Irradiation (250 Gy) of mass-reared moths had no influence ($F_{1,24} = 0.13$, $P = 0.72$) on their response to pheromone lures in flight-tunnel assays (Fig. 1). There was no interaction between temperature and irradiation treatment ($F_{1,24} = 0.10$, $P = 0.754$) indicating the effects of irradiation were the same at temperatures typical of spring (16 °C) and summer (25 °C) (Fig. 1). Temperature had a highly significant effect ($F_{1,24} = 102.69$, $P < 0.001$) on catches of mass-reared moths in this experiment (Fig. 1) and its effects were studied in more detail in subsequent experiments.

Experiment 2: effects of air temperature on pheromone response. For illustrative purposes the percentages of mass-reared moths caught in two separate tests were plotted against the complete range of temperatures evaluated in these tests (Fig. 2A). This plot suggests that the pheromone response × temperature function of mass-reared moths between 13 and 25 °C is nonlinear, but within the range of 15.5 and 19 °C it appears linear. Nonlinearity at higher temperatures is probably an experimental artifact because catches can not be greater than 100% and maximum response appears to have been reached near 19 °C (Fig. 2A). Nonlinearity at lower temperatures is an indication of a lower-temperature threshold for pheromone-mediated flight. This lower-temperature threshold seems most relevant within the context of comparing pheromone-mediated flight of mass-reared and wild codling moths and S:W trap-catch ratios.

Catches of wild codling moths in flight-tunnel assays were lower than mass-reared moths at every temperature tested (Fig. 2B). No wild moths were caught at 15 °C and only 58% were caught at 25 °C (Fig. 2B), compared with 85% catch of mass-reared moths (Fig. 2A). Linear regression was used to compare the temperature response function of the two populations of moths in flight-tunnel assays (Fig. 2B). The slopes (20.3 SIR *vs.* 5.6 Wild) of these regression lines were significantly different (*t*-test, t =

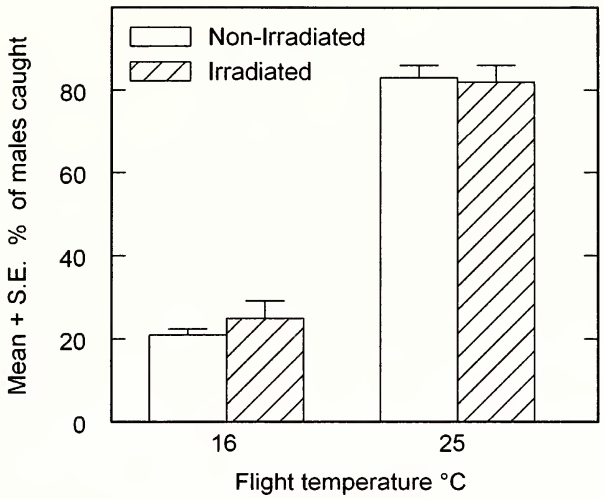

Figure 1. Mean + S.E. percentages of irradiated (250 Gy) and non-irradiated, mass-reared male codling moths caught in a synthetic pheromone-baited trap (red septum with 10 µg load) in 30-min flight-tunnel tests conducted at temperatures typical of spring (16 °C) and summer (25 °C). Two-way ANOVA indicates a significant temperature effect ($F_{1, 24} = 102.69$, $P < 0.001$) but no significant radiation effect ($F_{1, 24} = 0.13$, $P = 0.72$).

7.05, df = 26, $P < 0.001$). Similar *x*-intercepts suggest the lower-temperature thresholds for pheromone-mediated flight of wild (15.4 °C) and mass-reared males (14.7 °C) are similar (Fig. 2B) but no statistical test was made. The lower threshold for mass-reared moths was substantiated by our separate comparison of the percentages of mass-reared moths caught at 13, 14, 14.5 and 15 °C. In this experiment there was a significant difference in catches at 15 °C (16.3 ± 3.7%) and all other temperatures ($F_{3, 18} = 9.87$, $P < 0.001$), but not between 14.5 °C (5.7 ± 1.5%) and all lower temperatures (SNK test, $P < 0.05$).

Experiment 3: effects of handling time in moth release vehicles. Tests examining the pheromone response of mass-reared moths at different temperatures after being carried on an ATV delivery vehicle revealed a significant temperature effect ($F_{1, 36} = 87.44$, $P < 0.001$), but no significant handling time effects ($F_{1, 36} = 0.33$, $P = 0.57$) and no significant interaction between temperature and handling times ($F_{1, 36} = 0.98$, $P = 0.33$). However, when flown at 16 °C, the percentage of moths making contact with a pheromone lure after experiencing 4 h on an ATV was clearly depressed relative to contacts made by moths that spent no time on the ATV (Fig. 3). A statistical comparison isolating these two treatments found a significant reduction (two sample *t*-test, t = 2.62, df = 18, $P = 0.022$) as a result of being carried on the ATV that was not detected in moths flown at 25 °C (t = 0.67, df = 18, $P = 0.95$) (Fig. 3).

Field Tests

Experiment 4: effects of irradiation on pheromone trap catches of mass-reared and wild moths in the field. Irradiation significantly ($F_{1, 8} = 15.53$, $P = 0.004$) reduced recapture of both mass-reared and wild codling moths relative to non-irradiated moths in a field test conducted in late September (Fig. 4). Overall catches of mass-reared and wild moths in pheromone traps were not significantly different ($F_{1, 8} = 0.46$, $P = 0.517$). The effects of irradiation were independent of moth type ($F_{1, 8} = 0.02$, $P = 0.88$). Irradiated, mass-reared moths were about 1.5× less responsive to pheromone traps in this field test than were the non-irradiated wild moths they compete with in a sterile insect programme (Fig. 4).

Experiments 5 - 7: effects of moth release location on recapture. In two separate non-replicated releases conducted in mid September, irradiated, mass-reared

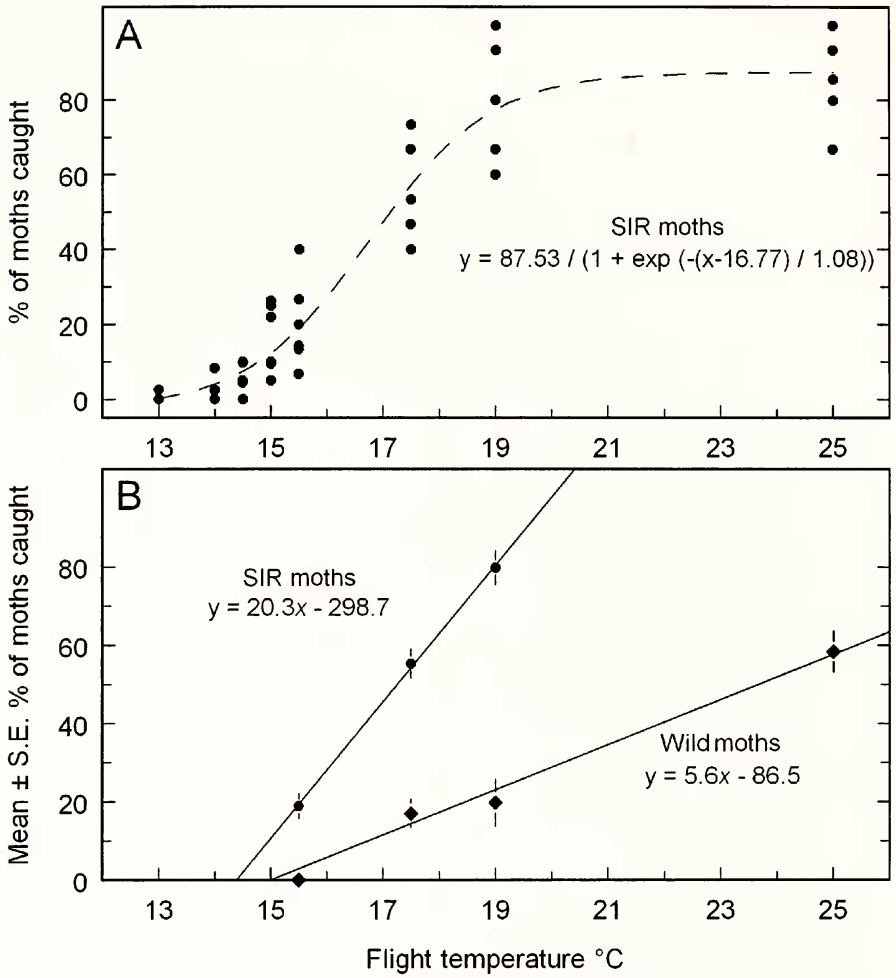

Figure 2. (A) Composite scatter plot of percentages of non-irradiated, mass-reared male codling moths (SIR moths) caught in a synthetic pheromone-baited trap (red septum with 10 µg load) in separate 30-min flight-tunnel tests conducted at different temperatures (13 - 25 °C). Dotted curve represents best-fit nonlinear regression line. (B) Plot of mean ± S.E. percentages of non-irradiated, mass-reared (SIR moths) and wild codling moths caught over temperature ranges of 15.5 - 19 °C for SIR moths and 15.5 - 25 °C for wild moths. Solid lines are best-fit least-squares linear regression lines for relationship between percentage catch and temperature (ANOVA n = 15; df =1, 13; $P < 0.001$ for each line). Slopes of regression lines are significantly different (*t*-test, t = 7.05, df = 26, $P < 0.001$).

moths released on the ground were recaptured (p_{GROUND}) significantly less often than canopy-released moths (Expt. 5: $p_{GROUND} = 0.227$ *vs.* $p_{CANOPY} = 0.435$; n = 128 moths released on ground and in canopy; $z = 3.2$, $P < 0.001$; Expt. 6: $p_{GROUND} = 0.422$ *vs.* $p_{CANOPY} = 0.601$; n = 128 moths released on ground and in canopy, $z = 2.5$, $P = 0.012$).

In replicated paired canopy and ground releases made during four weekly test periods (Fig. 5), moths released in the canopy were caught significantly more often than those released on the ground in weeks two, three and four, respectively ($t_1 = 4.19$, df = 3, $P = 0.025$; $t_2 = 15.57$, df = 3, $P = 0.001$; $t_3 = 4.81$, df = 3, $P = 0.017$). The four-week grand mean recapture rates for canopy- and ground-released moths in spring were 15.7 ± 2.4 and 4.9 ± 1%, respectively (Fig. 5).

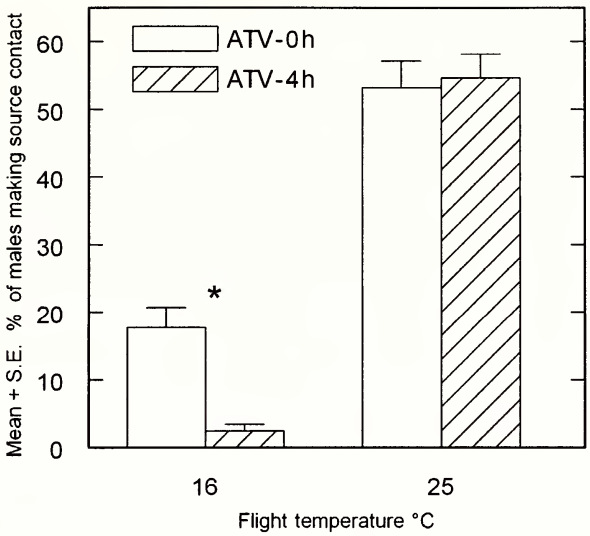

Figure 3. Mean + S.E. percentages of irradiated (250 Gy), mass-reared male codling moths contacting a synthetic pheromone lure (red septum with 10 µg load) in 2-min flight-tunnel tests conducted at temperatures typical of spring (16 °C) and summer (25 °C) after being carried for different times (0 *vs.* 4 h) on an all-terrain moth delivery vehicle (ATV). Two-way ANOVA indicates a significant temperature effect ($F_{1, 36} = 87.44$, $P < 0.001$) but no significant effect of time on an ATV ($F_{1, 36} = 0.33$, $P = 0.57$). Paired bars within a temperature grouping having an asterisk superscript are significantly different (t-tests, $P < 0.05$).

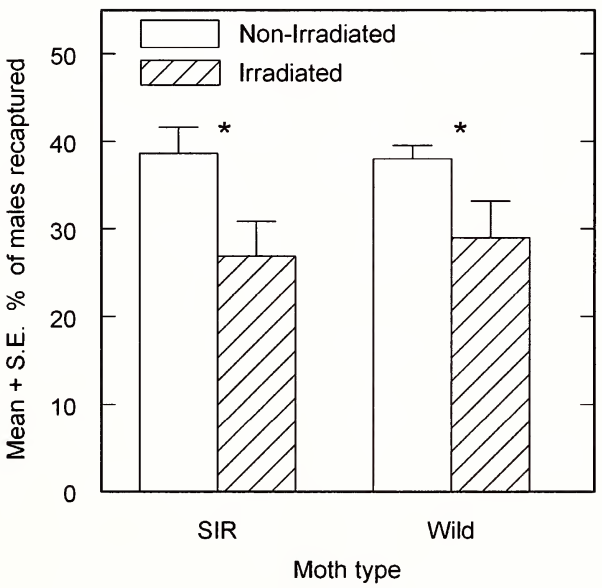

Figure 4. Mean + S.E. percentages of irradiated (250 Gy) and non-irradiated, mass-reared (SIR moths) and wild male codling moths recaptured in pheromone-baited (red septum with 10 µg load) Pherocon 1-CP wing traps after release in four apple orchards, Summerland, BC. Two-way ANOVA indicates a significant radiation effect ($F_{1, 8} = 15.53$, $P = 0.004$) but no significant moth effect ($F_{1, 8} = 0.46$, $P = 0.517$). Paired bars within a moth type having an asterisk superscript are significantly different (t-tests, $P < 0.05$).

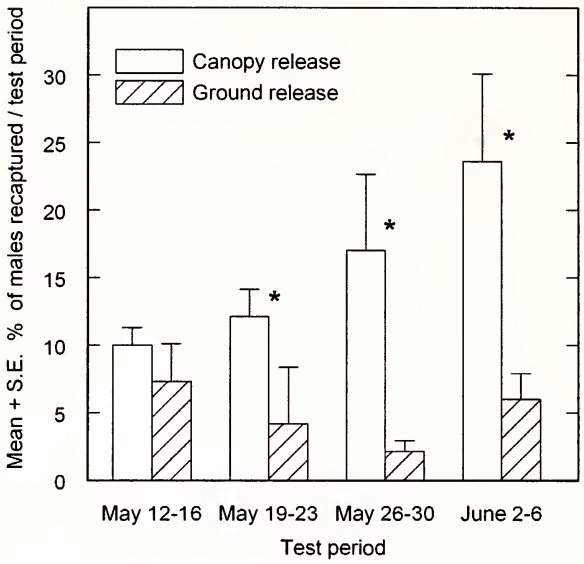

Figure 5. Mean + S.E. percentages of irradiated (250 Gy), mass-reared male codling moths recaptured in synthetic pheromone-baited traps (red septum with 10 μg load) after release on the ground or within the canopy of apple orchards, Summerland, BC. Paired bars within each weekly test period having an asterisk superscript are significantly different (t-tests, $P < 0.05$).

DISCUSSION

Our examination of flight activity and recapture of sterile, mass-reared moths released by the Okanagan-Kootenay SIR Programme has helped identify factors that contribute to low activity of sterile moths in spring (Judd *et al.* 2004). Determining the impact of various factors in a complex operational SIR Programme is challenging because many factors interact and vary irregularly across orchards and seasons. Even in controlled studies like those conducted here interpretation requires careful consideration.

Irradiation with 250 Gy did not appear to impair pheromone perception and behavioural response when moths were assayed under controlled laboratory conditions. In flight-tunnel assays, equal proportions of irradiated and non-irradiated mass-reared moths were caught in traps baited with 10 μg pheromone lures (Fig. 1). Although catches with pheromone lures were lower at 16 than at 25 °C, there was no significant difference in the proportions of irradiated and non-irradiated moths caught at each of these temperatures, respectively (Fig. 1). This lack of a significant irradiation effect on catches with pheromone traps in laboratory assays is both supported and contradicted by field studies. Bloem *et al.* (1999) made several field releases in late June, July and August and found that mass-reared moths irradiated with 250 Gy were recaptured in female-baited traps at the same rate as non-irradiated moths. Likewise, Judd *et al.* (2006a) conducted mark-release-recapture experiments in May and August and found that mass-reared moths irradiated with 250 Gy were recaptured in standard monitoring traps loaded with 1-mg codlemone lures as often as non-irradiated mass-reared moths. However, in the September study reported here (Fig. 4), we found that irradiation did cause a reduction in recapture of both mass-reared and wild codling moths in traps baited with 10 μg lures.

The conclusion we draw from these various data sets is that small spring-time catches of sterile moths in pheromone traps is not caused by radiation-induced impairment of the olfactory system. If it was, this

very direct effect should show up consistently across laboratory and field tests, since the radiation treatment is the one factor that remains consistent across studies. It seems more likely that irradiation has an indirect effect on pheromone response, probably by reducing general flight activity and dispersal, which might reduce the frequency with which sterile moths encounter pheromone plumes in the field. When placed directly in pheromone plumes the irradiated moths appear as responsive to pheromone sources as do non-irradiated moths. If the effects of irradiation are mainly to reduce moth activity, then variable environmental test conditions that also affect activity, could easily explain the varied impact of irradiation in different field studies. The other factor that could come into play in different studies is the quality of the non-irradiated moths used in comparison with irradiated moths. Judd *et al.* (2006a) found that non-irradiated mass-reared moths were recaptured ca. 4× less often than non-irradiated wild moths released under identical conditions, and S:W trap-catch ratios were as low as those observed by the SIR Programme in the spring. The recapture of non-irradiated moths was so poor in that study that irradiation contributed little effect. The effects of irradiation on the activity of sterile moths in the spring are obviously complicated by external interactions not yet fully understood.

Temperature had a significant effect on the response of codling moths to pheromone lures in all flight-tunnel assays (Figs. 1, 2 & 3), however, the hypothesis that mass-reared moths fly less frequently at low temperatures than wild moths was not supported by our data (Fig. 2). Our estimated lower-temperature threshold for pheromone response of mass-reared codling moths was 14.7 °C, while the established lower-temperature threshold for pheromone trap catches of wild codling moths in the field is 15.6 °C (Reidl *et al.* 1986) and for wild codling moths in flight-tunnel assays it was 15.4 °C (Fig. 2). Therefore, an inability of sterile moths to engage in pheromone-mediated flight at low temperatures is probably not responsible for their low catches in pheromone traps during spring.

While we were unable to demonstrate any effect of mass-rearing on temperature thresholds for pheromone-mediated flight, our assays would not necessarily detect differences in the response of wild and mass-reared moths to temperature transitions that are common in the field. In spring, temperatures often decline very quickly before the normal dusk flight period (Judd *et al.* 2006a,b). These temperature transitions stimulate an earlier release of pheromone by female codling moth (Castrovillo and Cardé 1979) and an earlier male response to pheromone (Batiste *et al.* 1973, Song and Reidl, 1985). Judd *et al.* (2006a) demonstrated that during temperature transitions wild male codling moths mated significantly earlier than mass-reared moths, suggesting wild moths have an earlier or quicker response to pheromone while temperatures are declining.

Even if mass-rearing has no affect on temperature thresholds for pheromone-mediated flight, it could be affecting temperature thresholds for general activity or dispersal from release locations. While the mass-rearing system currently used by the SIR Programme incorporates flight of moths as part of its collection and rearing process, this flight occurs in response to UV light at 27 °C (Bloem and Bloem 2000). Moths that do not respond well to UV light or are inactive at 27 °C are excluded from the rearing system because they never get collected. This collection system could inadvertently select for different activity thresholds. It would be interesting to compare the response of wild and mass-reared moths to UV lights at temperatures closer to the pheromone-mediated flight threshold in order to determine whether mass-reared moths have undergone more general changes in activity. Differences of this type between wild and mass-reared moths could be an important factor causing low S:W ratios and deserves examination.

While there may not be an obvious difference in the flight-temperature threshold for wild and mass-reared moths, the tem-

perature profiles to which they are both exposed in the field is likely quite different. Many wild moths emerge on the bark within the canopy of host trees, and ambient temperatures on the bark are often greater than air or ground temperatures (unpublished data). Unlike wild moths which warm naturally as part of a temperature-regulated emergence process, sterile moths are chilled, up to 48 h in some cases, before being dispensed onto cold ground. Some moths spend an additional 4 h in a cooler on an ATV before being dispensed. Moths that were carried on an ATV for 4 h were somewhat less responsive to pheromone in flight-tunnel assays conducted at spring temperatures than were moths not carried on the ATV (Fig. 3). In field experiments conducted in the spring, ground-released moths were recaptured ca. 3× less often than canopy-released moths (Fig. 5). It seems plausible therefore that ground delivery of chilled moths in combination with cool soil temperatures is contributing significantly to reduced flight activity of sterile moths in spring. If sterile moths released in the spring spend a greater period of time on the ground than those in summer, they may be more susceptible to predation. Predation could significantly reduce the effective number of sterile moths flying up into the orchard canopy. The degree to which sterile moths are preyed upon and seasonal differences in predation rates have not been studied but probably should be.

Producing good quality insects is obviously one of the most important components of a robust sterile insect programme (Huettel 1976). Over time the Okanagan-Kootenay SIR Programme has made a number of improvements in moth quality by shortening the time moths spend in cold storage before being shipped to the field and decreasing the time moths spend on ATVs. The programme has also reduced radiation doses from the original 350 to 250 Gy (Dyck *et al.* 1993, Bloem and Bloem 2000). Nevertheless, spring S:W ratios in this operational programme have remained far below the 40:1 target ratio even though the numbers of sterile moths being released has increased over time. Because of increasing costs, inadequate S:W ratios and slower than expected population declines, the use of sterile moths as a management tool for area-wide control of codling moth in BC is subject to continuing discussion (Dendy *et al.* 2001, Thistlewood and Judd 2003). Based on current technology we recently concluded that the most effective use of sterile moths in an area-wide codling moth control programme was to restrict delivery to summer and augment control by applying other tactics in spring (Judd and Gardiner 2005). Results presented here suggest that significant improvements in the quality of sterile moths and increases in S:W ratios might be gained by changing the delivery system. Aerial release seems a logical alternative but has its own difficulties in a highly-urbanized mountainous valley system. Development of a ground delivery process which limits time in cold storage, minimizes moth damage (such as loss of wing scales) while being carried on ATVs, and dispenses moths into the canopy rather than onto the ground, should probably be considered. If the effects of a ground-delivery system are not considered and addressed, then expected improvements in the quality of mass-reared moths gained by modifying the rearing system (Judd *et al.* 2006b) might never be realized.

ACKNOWLEDGEMENTS

We thank the Similkameen-Okanagan Organic Producers' Association and its cooperating members for allowing us to conduct trials in their orchards, and Nicole Weremy for her technical assistance. This research was funded by the Similkameen-Okanagan Organic Producers' Association, Washington State Tree Fruit Research Commission, and the Agriculture and Agri-Food Canada Matching Investment Initiative.

REFERENCES

Batiste, W.C., W.H. Olson, and A. Berlowitz. 1973. Codling moth: influence of temperature and daylight intensity on periodicity of daily flight in the field. Journal of Economic Entomology 66: 883-892.

Bäckman, A.-C. 1997. Pheromone release by codling moth females and mating disruption dispensers. IOBC WPRS Bulletin 20: 175-180.

Bloem, K.A. and S. Bloem.1996. Codling moth eradication program in British Columbia: a review and update, pp. 101-110. *In* M.T. Ali Niazee and L.E. Long (eds.), Biology and control of cherry fruit flies: a worldwide perspective. Special Report 971. Oregon State University, Agricultural Experiment Station, Corvallis, Oregon.

Bloem, K.A. and S. Bloem. 2000. SIT for codling moth eradication in British Columbia, Canada, pp. 207-214. *In* K.-H. Tan (ed.), Area-wide control of fruit flies and other insect pests. Joint Proceedings of the International Conference on area-wide control of insect pests, and the Fifth International Symposium on fruit flies of economic importance, May-June 1998. Penerbit Universiti Sains Malaysia, Pulau Penang.

Bloem, S., K.A. Bloem, and A.L. Knight. 1998. Assessing the quality of mass-reared codling moths (Lepidoptera: Tortricidae) by using field release-recapture tests. Journal of Economic Entomology 91: 1122-1130.

Bloem, S., K.A. Bloem, J.E. Carpenter, and C.O. Calkins. 1999. Inherited sterility in codling moths (Lepidoptera: Tortricidae): effect of substerilizing doses of radiation on field competitiveness. Environmental Entomology 28: 669-674.

Bloem, S., J.E. Carpenter, K.A. Bloem, L. Tomlin, and S. Taggart. 2004. Effect of rearing strategy and gamma radiation on field competitiveness of mass-reared codling moths (Lepidoptera: Tortricidae). Journal of Economic Entomology 97: 1891-1989.

Castrovillo, P.J. and R.T. Cardé. 1979. Environmental regulation of female calling and male response periodicities in the codling moth (*Laspeyresia pomonella*). Journal of Insect Physiology 25: 659-667.

Brinton, F.E., M.D. Proverbs, and B.E. Carty. 1969. Artificial diet for mass production of the codling moth, *Carpocapsa pomonella* (Lepidoptera: Olethreutidae). The Canadian Entomologist 101: 577-584.

Dendy, C., M.G. Powell, and Associates. 2001. A study of the financial sustainability of the Okanagan-Kootenay SIR Program for control of codling moth post 2005. Report to the Okanagan Valley Tree Fruit Authority and Okanagan-Kootenay SIR Board. Vernon, BC. August 2001.

Dyck, V.A., S.H. Graham, and K.A. Bloem. 1993. Implementation of the sterile insect release programme to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), in British Columbia, Canada, pp. 285-297. *In* IAEA Management of Insect Pests: Nuclear, and Related Molecular Techniques, Vienna, 1992. SM-327/29.

Huettel, M.D. 1976. Monitoring quality of laboratory reared insects: a biological and behavioural perspective. Environmental Entomology 5: 807-814.

Hutt, R.B. 1979. Codling moth (*Laspeyresia pomonella* (Lepidoptera: Olethreutidae)): improving field performance of mass-reared males. The Canadian Entomologist 111: 661-664.

Judd, G.J.R., M.G.T. Gardiner, and D.R. Thomson. 1997. Control of codling moth in organically-managed apple orchards by combining pheromone-mediated mating disruption, post-harvest fruit removal and tree banding. Entomologia Experimentalis et Applicata 83: 137-146.

Judd, G.J.R., M.G.T. Gardiner, and H.M.A. Thistlewood. 2004. Seasonal variation in recapture of mass-reared sterile codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae): implications for control by sterile insect technique in British Columbia. Journal of the Entomological Society of British Columbia 101: 29-43.

Judd, G.J.R. and M.G.T. Gardiner. 2005. Towards eradication of codling moth in British Columbia by complimentary actions of mating disruption, tree banding and sterile insect technique: five-year study in organic orchards. Crop Protection 24: 718-733.

Judd, G.J.R., M.G.T. Gardiner, N.C. DeLury, and G. Karg. 2005. Reduced antennal sensitivity, behavioural response and attraction of male codling moths, *Cydia pomonella* (L.), to their pheromone (*E*, *E*)-8,10-dodecandien-1-ol following various pre-exposure regimes. Entomologia Experimentalis et Applicata 114: 65-78.

Judd, G.J.R., H.M.A. Thistlewood, M.G.T. Gardiner, and B.L. Lannard. 2006a. Is lack of mating competitiveness in spring linked to mating asynchrony between wild and mass-reared male codling moth from an operational sterile insect programme? Entomologia Experimentalis et Applicata 120: 113-124.

Judd, G.J.R., S. Cockburn, C. Eby, M.G.T. Gardiner, and S. Wood. 2006b. Diapause improves spring-time mating competitiveness of male codling moth mass-reared for a sterile insect programme. Entomologia Experimentalis et Applicata 120: 161-166.

McMechan, A.D. and M.D. Proverbs. 1972. Equipment and procedures for release of sterile codling moths. Canadian Agriculture Engineering 14: 42-45.

Proverbs, M.D., J.R. Newton, and C.J. Campbell. 1982. Codling moth: a pilot program of control by sterile

insect release in British Columbia. The Canadian Entomologist 114: 363-376.

Reidl, H., J.F. Howell, P.S. McNally, and P.H. Westigard. 1986. Codling moth management: use and standardization of pheromone trapping systems. Agricultural Experiment Station, Division of Agriculture and Natural Resources, University of California. 2.5m-pr-9/86-HS/ME.

Rogers, D.L. and C.J. Winks. 1993. Quality control in laboratory-reared codling moth at Mt. Albert Research Centre, Auckland, New Zealand, pp. 13-21. *In* G. Nicoli, M. Benuzzi and N.C. Leppla (Eds.), Global IOBC working group "Quality Control of Mass-Reared Arthropods", Proceedings of the 7th workshop in Rimini, Italy, September 13-16, 1993.

Song, Y.-H. and H. Riedl. 1985. Effects of temperature and photoperiod on male activity in *Laspeyresia pomonella* (L.) in New York. Korean Journal of Plant Protection 24: 71-77.

Thistlewood, H.M.A. and G.J.R. Judd. 2003. Area-wide management of codling moth, *Cydia pomonella*, at very low densities. IOBC WPRS Bulletin 26: 103-109.

Thistlewood, H.M.A., G.J.R. Judd, and M.E.O. Clodius. 2004. Sustainable management and monitoring of codling moth, *Cydia pomonella*, in an area-wide program employing SIT, pp. 79-87. *In* IAEA (Ed.), Improvement of Codling Moth SIT to Facilitate Expansion of Field Application, Stellenbosch, South Africa, March 8-12, 2004. Working Paper Series. International Atomic Energy Agency, Vienna, Austria. IAEA-314-D4-RC876.

Zar, J.H. 1984. Biostatistical Analysis, Second edition. Prentice Hall, Englewood Cliffs.